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Cite this: *Med. Chem. Commun.*, 2018, **9**, 893

Elucidation of fluorine's impact on pK_a and *in vitro* Pgp-mediated efflux for a series of PDE9 inhibitors†

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Received 28th February 2018,
Accepted 15th April 2018

DOI: 10.1039/c8md00114f

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P-Glycoprotein (Pgp)-mediated cellular efflux is recognized as a common challenge in CNS drug discovery. In this study, the influence of replacing a hydrogen atom with fluorine on the pK_a and Pgp-mediated efflux is elucidated for a series of PDE9 inhibitors. The PDE9 inhibitors with and without fluorine were synthesized using a novel condensation–oxidation approach, providing access to several analogues, all from the same stereoenriched aldehyde building block. The incorporation of fluorine was found to influence two acid–base functionalities concomitantly, both of which were involved in Pgp-recognition. By methylating the acidic functionality, it was possible to isolate the effect responsible for lowering the Pgp-mediated efflux.

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1. Introduction

Medicinal chemists involved in drug discovery increasingly appreciate the changes of key properties afforded by the introduction of fluorine in their lead compounds. This remarkable element has been shown to affect the electron density of neighboring functional groups, altering the compound's pK_a, conformation, solubility, permeability and binding affinity.[1–4] Additionally, fluorine has also been used in strategic replacements for metabolically liable hydrogen atoms, improving half-life significantly.[5,6] Introducing fluorine to modulate the basicity of simple aliphatic amines generally results in a significant shift in pK_a, if installed at either the β or γ-position.[7,8] In the area of CNS drug design, among the most crucial parameters to optimize are passive permeability and Pgp-mediated efflux, and to de-risk complications associated with poor brain exposure, the efflux ratio should generally be <2.5.[9,10] To control a compound's Pgp-recognition, of particular relevance to medicinal chemists are the hydrogen bonding capabilities, log *D*, topological polar surface area (tPSA) and basicity.[11,12] For a chemical series with a consistent number of hydrogen bond donors/acceptors, the individual H-bond strengths become particularly important. Thus, being able to introduce a fluorine atom at a specific position can play a pivotal role in modulating these core parameters without drastically altering molecular size. Encouraged by our recent diastereodivergent fluorination of branched pyrrolidinyl carbaldehydes,[13] we set out to study the influence of H/F replacement on the activity, pK_a and Pgp-mediated efflux of a series of phosphodiesterase 9 (PDE9) inhibitors (Fig. 1).

These compounds containing the 3,4-substituted pyrrolidine scaffold constitute good candidates for such examination, as the cellular flux ratios within the series vary significantly depending on the nature of the R group and is thus likely to be effected by fluorine.[14] The PDE enzymes are involved in the hydrolysis and break-down of the cyclic nucleotides cAMP and cGMP that serve as key intracellular initiators of numerous signaling processes in the nervous system. Of all the PDE enzyme families, PDE9 has the highest affinity for cGMP, and inhibition of the PDE9 enzyme to increase cGMP levels has been reported to enhance cognitive function in rodents.[15,16] The 3,4-substituted pyrrolidine-based PDE9 inhibitors in Fig. 1 without fluorine were originally discovered by Pfizer,[14] and the pyrimidinylated analogue PF-

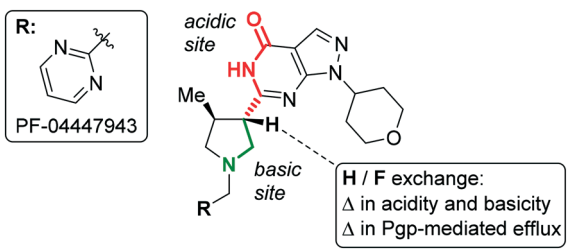

Fig. 1 Series of PDE9 inhibitors. R = aromatic or heteroaromatic group.

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† Electronic supplementary information (ESI) available: Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra, and details of the assay used for determination of MDCK-MDR1 ratios and pK_a's. See DOI: 10.1039/c8md00114f

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04447943 was advanced into phase 2 trials to assess its application in mild to moderate probable Alzheimer's Disease treatment.

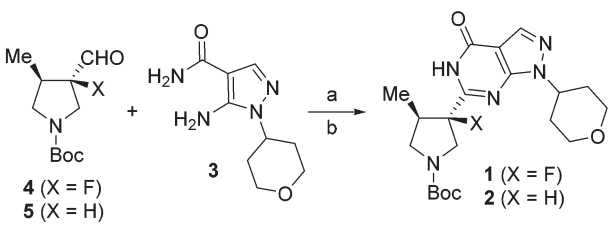
As will become evident from this study, introduction of fluorine had a moderate effect on PDE9 inhibition of these compounds – PDE9 IC50 values varied from unchanged to a 10-fold increase (*i.e.* reduced potency). Contrary to our expectations, the introduction of fluorine led to a significant increase in Pgp mediated efflux in some cases – probably due to increased acidity of the amide moeity. In other cases where the acidic site was blocked by methylation, the Pgp mediated efflux was reduced by fluorination in the β-position with regard to the amine moiety.

2. Chemistry

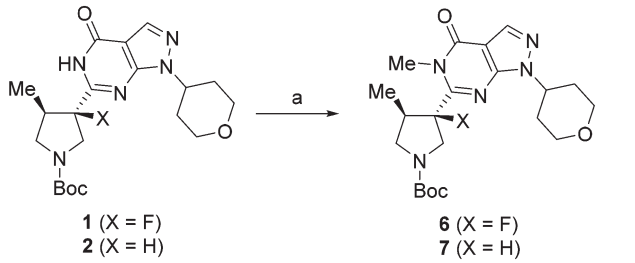
Synthetic access to the fluorinated PDE9 inhibitor analogues in a convenient manner relied on the formation of a common fluorinated intermediate **1** and the non-fluorinated **2** that could be converted directly into the final products (Scheme 1). Inspired by Verhoest *et al.*, a pyrazolyl amide building block **3** could be condensed with a carbonyl derivative to furnish the pyrazolopyrimidinone core.[14] We hypothesized that a β-fluoropyrrolidinyl carbaldehyde **4**, prepared by stereoselective organocatalysis could be used as starting point for the condensation–oxidation approach with **3** to directly provide the bicyclic core. This strategy was based on findings by Togo *et al.* in which ethylenediamine was condensed with a series of aldehydes and oxidized to the corresponding imidazolines using molecular iodine and potassium carbonate.[17] This direct approach furnished the desired common intermediate **1** in 14% yield along with a range of byproducts. Further optimization led to the use of $Ti(OEt)_4$ to promote condensation followed by filtration and oxidation with I_2 at 80 °C for 30 min. Thus, the yield increased to 36% for **1** and the protocol also successfully transformed the non-fluorinated aldehyde **5** into **2** in 56% yield (Scheme 1).

Having established a route to **1** and **2**, the corresponding *N*-methylated lactams **6** and **7** could be prepared using NaH and MeI in DMF (Scheme 2).

The preparation of **1** and **2** allowed subsequent synthesis of a range of final products **8–11** using a two-step sequence in which the Boc-protecting group was removed using HCl in methanol followed by reductive amination with commercially



Scheme 1 Reagents and conditions: a: $Ti(OEt)_4$, THF (0.5 M), 75 °C, 24 h then filtration and b: I_2 (1.5 eq), DMF (0.15 M), 80 °C, 30 min. X = H (56%), F (36%).



Scheme 2 Reagents and conditions: (a) NaH, MeI, DMF, 0 °C, 1.5 h. X = H, (28%), F (13%).

available aldehydes (Table 1). To our satisfaction, the conversion of common intermediates **1**, **2**, **6** and **7** to the respective final products were successful and moderate yields were obtained for the two-step processes.

3. Results & discussion

The condensation–oxidation methodology (Scheme 1) combined with the reductive amination chemistry provided us with several candidates for *in vitro* examination, featuring only minor structural variance to aid isolating the impact of H/F replacement. The pK_a's for both the acidic and basic sites for compounds **8a–f** were experimentally determined. The acidic functionality found in the pyrazolopyrimidinone cores had pK_a's below 10, being even lower than phenol, indicating a strong hydrogen-bond capability for an N–H donor (Table 1)[18] The difference in pK_a between **9a** and its fluorinated analogue **8a** falls well within the interval expected for small cyclic amines. Similar comparisons through the complete list of compounds and their fluorinated analogues **8a–f** reveals a systematic decrease in acidic pK_a between 1.2–1.7 (pK_a (acidic)), and shifts for the basic pyrrolidines between 1.1–3.3 units (pK_a (basic)). Throughout the series **8–9**, the fluorinated analogues generally had lower potencies. In half the cases, the Pgp-efflux ratios were significantly affected by the presence of fluorine; for **8a**, **8b** and **8e**, the efflux ratios significantly increased, whereas they remained unchanged for **8c**, **8d** and **8f**. The introduction of fluorine simultaneously affects pyrrolidine-basicity and the lactam acidity-parameters most likely involved in either increasing or decreasing Pgp-recognition, respectively. For a structurally advanced scaffold as studied herein featuring several functional groups, the outcome of such opposing effects might be difficult to predict *a priori* or quantitatively rationalize posteriori. Thus, we were interested in isolating and evaluating the effect responsible for decreasing the efflux ratio. For this reason, we chose to study the pairs of *N*-methylated lactams **10e–f** and **11e–f** lacking the hydrogen bond donor. These compounds were chosen as both fluorinated non-methylated analogues **8e–f** had high flux ratios and thus, a potential decrease in Pgp-efflux would likely be noticeable.

Not surprisingly, a major loss of activity throughout the *N*-methyl series was observed, which is rationalized by the loss of a key hydrogen-bond interaction between the lactam and

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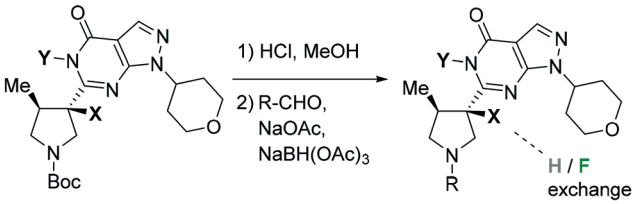
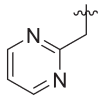
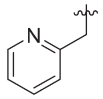
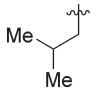
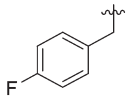
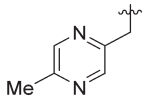
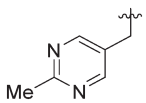
Table 1 Data for the fluorinated (**8**, **10**) and non-fluorinated PDE9 inhibitors (**9**, **11**)[a,b,c,d,e]



1, 2, 6, 7 **8-11**

Comp.	R group	X	Y	MDCK-MDR1 ratio	Δ	pK_a (acidic)	pK_a (basic)	IC_{50}[a] (PDE9)	Yield
9a		H	H	1.3	↑	9.6	6.8	49 nM	n/a
8a		F	H	6.9		8.4	5.6	200 nM	62%
9b		H	H	1.9	↑	9.6	7.0	14 nM	36%
8b		F	H	4.2		8.4	5.9	220 nM	47%
9c		H	H	1.3	≈	10.1	8.4	750 nM	33%
8c		F	H	0.9		8.4	5.9	590 nM	33%
9d		H	H	1.4	≈	9.7	7.9	17 nM	63%
8d		F	H	1.4		8.5	6.5	65 nM	40%
9e		H	H	2.7	↑	9.7	8.8	59 nM	50%
8e		F	H	7.1		8.4	5.5	180 nM	35%
11e		H	Me	3.1	↓	n/a	6.8	66% (10 μM)	55%
10e		F	Me	1.8		n/a	5.2	22% (10 μM)	48%
9f		H	H	15.5	≈	9.5	7.7	36 nM	33%
8f		F	H	16.0		8.4	4.9	210 nM	75%
11f		H	Me	4.7	↓	n/a	6.4	850 nM	59%
10f		F	Me	2.6		n/a	4.9	14% (10 μM)	48%

[a] IC_{50}: potency in the PDE9 assay, values in % correspond to the inhibition at 10 μM.[19] [b] MDCK-MDR1: the Madin Darby canine kidney permeability assay with a multi-drug resistant gene coding for Pgp over-expression. The efflux ratio is calculated as basolateral-apical/apical-basolateral. See ESI for further details.[20] [c] n/a = not applicable. [d] Δ reflects the direction of the change in MDCK-MDR1 efflux ratio after H/F exchange. [e] Compounds **9a**, **b**, **d**–**f** have previously been prepared through a different route.[14]

glutamine-453 in the binding pocket of the PDE9 enzyme.[14] Despite the expected loss in activity, further elucidation of how pK_a changes, within such structurally similar compounds, influence the Pgp-mediated efflux continued to pique our interest. The differences in pyrrolidine basicities were determined between each of the respective compounds **10e**–**f** and **11e**–**f** to be 1.5 and 1.6 pK_a units. It was particularly interesting that within this *N*-methyl lactam series, **10e**–**f** and **11e**–**f**, the Pgp-mediated efflux was significantly lowered from 3.1 to 1.8 and 4.7 to 2.6 for the fluorinated compounds, placing them into the acceptable region for CNS candidates. Thus, in support of our hypothesis that two opposing effects were affecting the MDCK-MDR1 ratio, removing the acidic functionality by methylation indeed led fluorine to decrease the Pgp-mediated efflux for both **10e** and **10f**. The prediction of how H/F replacement exactly impacts a compound's pharmacokinetic and pharmacodynamic properties is a complex matter. However, during the final stages of lead optimization in drug discovery, where the general molecular framework is established, possessing the synthetic means for introducing a fluorine atom can be a resourceful means to balance the properties. In addition, *e.g.* for biological targets in the periphery, it is desired to prevent the active compound from entering the brain. For compounds, similar to **8** and **9**, fluorine incorporation might even be a viable strategy to decrease brain exposure by increased Pgp-mediated efflux. However, prediction of fluorine's impact *a priori* might be simpler in cases where fluorine only affects either a hydrogen bond donor or acceptor for compounds facing the hurdle of Pgp-efflux.

Conclusion

In conclusion, several fluorinated and non-fluorinated drug-like compounds were synthesized from a single aldehyde **5** using a diastereoselective fluorination protocol to give **4**,[13] and followingly a novel condensation–oxidation approach to yield the common precursors **1**, **2** and **6**, **7**. These precursors were then converted into the final compounds **8**–**11** that were used to elucidate the impact of fluorine incorporation on pK_a and Pgp-mediated efflux. The consistent trend observed in the pK_a shifts for **9** and **11** after H/F replacement to give **8** and **10**, and the relationship between pK_a, the presence of a

hydrogen bond donor and the efflux ratio aid the general understanding of the possibilities and limitations afforded by fluorine in drug discovery. Future strategies in CNS drug design may benefit from such findings towards a more effective optimization process in the search for compounds that can successfully progress towards clinical candidate status.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

The work was financially supported by the Ministry of Higher Education and Science and H. Lundbeck A/S. We would like to also thank Dr. Henrik Pedersen from H. Lundbeck A/S with analytical assistance in HR MS acquisition.

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20 Provided by WuXi AppTec, Shanghai, China.

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